

Attorney Docket No.:	DC0266US.NP
Inventors:	Kitareewan, et al.
Serial No.:	10/564,070
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#### REMARKS

Claim 8 is pending in this application. Claim 8 has been rejected. Claim 8 has been amended. No new matter has been added by this amendment. Reconsideration is respectfully requested in light of the following remarks.

#### I. Rejection Under 35 U.S.C. §103

Claim 8 has been rejected under 35 U.S.C. 103(a) as being unpatentable over Bard et al. (1977) in view of Yoshida et al. (1996). The Examiner suggests that Bard et al. teaches that "at higher than physiological concentrations" retinoids destabilize membranes causing the release of lysosomal enzymes and the effect can be followed by metachromatic staining and by measuring the appearance of proteoglycan fragments in the medium of organ cultures of rabbit ear cartilage. The Examiner acknowledges that this reference fails to teach a method comprising contacting a cell expressing PML/RAR $\alpha$  and detecting whether said agent destabilizes lysosomes of the cell as determined by vital staining of lysosomes or release of lysosomal proteins into the cytosol, as well as increasing lysosomal-dependent PML-RAR $\alpha$  degradation. However, the Examiner suggests it would have been obvious for one of ordinary skill in the art to combine the teaching of Bard et al. with the teaching of Yoshida et al. (1996) where Yoshida et al. teaches contacting an APL cell that expresses PML/RAR $\alpha$  with an anti-cancer agent, specifically ATRA, to arrive at the method of the present invention. The Examiner suggests that one of skill would have been aware that as lysosomes destabilize they release lysosomal enzymes into the cytosol which would actively degrade any proteins they encounter, including

PML/RAR $\alpha$ . Thus, the Examiner suggests that the limitation that the protein be "lysosomal-dependent" is inherently met as destabilization of the lysosomes releases enzymes and would not otherwise occur, making the resulting degradation necessarily lysosomal dependent. Applicants respectfully traverse this rejection.

At the outset, in an earnest effort to advance the prosecution and facilitate allowance of the claim, Applicants have amended claim 8 to recite that the method of the present invention involves contacting a cell that expresses PML/RAR $\alpha$  with an "effective amount of an" agent as well as stating that "an effective amount of said agent is a concentration that is clinically useful". Support for this amendment can be found throughout the specification as filed but in particular at page 15, lines 13-23, where an effective amount of an agent is defined as one that is in an amount sufficient to effect beneficial or desired results, including "clinical results".

Bard *et al.* (1977), as acknowledged by the Examiner, discloses investigations into the toxicity of retinoids in a rabbit model and understanding the mechanism of retinoid-induced toxicity in tissues such as cartilage. The retinoids were administered at doses of from 2 to 20  $\mu$ M, doses or concentrations that one of skill in the art would know produce toxicity, not doses that are clinically useful since induction of toxicity is an undesired clinical result. In fact, this specific issue of undesirable toxicity and its role in development of clinically useful anti-cancer drugs is discussed by Bard *et al.* at page 118 where it is stated:

"The results support the hypothesis that retinoic acid may act as a detergent in destabilizing lysosomal

*membranes* (Dingle and Fell, 1963), since all the compounds with carboxylic acid groups are active, and the replacement of the acid moiety by less hydrophilic groups would diminish their efficiency as surfactants. The results also imply that in cartilage, at least, ethers, esters and amides are not converted extensively to retinoic acid. The possibility that these conversions may take place in other tissues, and consequently affect the pharmacology of these compounds, cannot be excluded at present.

The anti-carcinogenic activity appears to be unrelated to the interaction with membranes, since the most effective anti-carcinogens are not necessarily the most polar (Lasnitzki, 1976), and detergents of different structures do not oppose tumour development (Lasnitzki and Goodman, 1974). The identification of compounds which combine potent anti-carcinogenic action with low activity in destabilizing lysosomes offers considerable hope for the eventual development of an effective anti-tumour agent of low general toxicity.[emphasis added]"

Clearly, Bard et al. teach that retinoic acid itself possess a toxicity profile that involves lysosomal destabilization and that such lysosomal destabilization is not an attribute that would be sought by one of skill in the art when developing retinoic acid analogs as anti-cancer drugs, or in screening drugs for ability to inhibit cancer cell growth, such as is claimed in the instant invention.

"A reference may be said to teach away when a person of ordinary skill, upon reading the reference, would be discouraged from following the path set out in the reference, or would be led in a direction divergent from the path that was taken by the applicant." *In re Gurley*, 27 F.3d 551, 553 (Fed. Cir. 1994). Bard et al. clearly teach away

from the method of the present invention which relies on the ability of an agent to destabilize lysosomes within a cell as a desired drug effect. Thus, the reference of Bard et al. fails to teach or suggest all limitations of the instant claims but also teaches away from the instant method and as such would not provide one of skill with either a motivation to combine this teaching with other prior art or to expect success when combining the teaching with other art to arrive at a method of identifying agents that could be used clinically to treat cancer.

Yoshida et al. (1996), when combined with Bard et al. (1977) fails to teach the limitations of the claim as filed and also as now amended. In previous replies filed during prosecution of this case, Applicants respectfully pointed to the paragraph spanning pages 2946-2947 of Yoshida et al. which states:

Accordingly, we supposed that PML-RARA bound with ATRA might become unstable and undergo accelerated degradation. The degradation of most cellular proteins is catalyzed by the nonlysosomal ubiquitin-proteasome pathway, which is dependent on ATP and closely involved in the proteolysis of aberrantly generated products (13). To investigate whether proteasomes are involved in the decrease of PML-RARA by ATRA, we examined the effect of the *Streptomyces* metabolite lactacystin (14), a highly specific inhibitor of the proteasome (15). The decrease of PML-RARA induced by ATRA was dose-dependently inhibited by lactacystin (Fig. 4). Lactacystin at 10  $\mu$ M almost completely inhibited the decrease of PML-RARA induced by 1  $\mu$ M ATRA. These data suggest that ATRA accelerates the degradation of PML-RARA in the proteasome pathway.

Therefore, the cited passage specifically teaches that "ATRA accelerates the degradation of PML-RAR $\alpha$  in the proteasome

pathway." This is in direct contrast to the instant invention, which is directed to the identification of agents that induce the lysosome-dependent degradative pathway (page 5, lines 1-26 of the instant specification). Indeed, as described at page 5, lines 10-11, proteasome and caspase inhibitors do not block PML/RAR $\alpha$  degradation in accordance with the instant assay. In this respect, the instant assay requires the identification of agents that both destabilize lysosomes and increase PML/RAR $\alpha$  protein degradation. It is only with the specification in hand that one of skill would be motivated to consider developing a method as claimed which involves contacting a cell that expresses PML/RAR $\alpha$  with an effective amount of an agent, wherein an effective amount of said agent is a concentration that is clinically useful, and detecting whether said agent first destabilizes lysosomes of the cell, and second increases lysosomal-dependent PML/RAR $\alpha$  protein degradation.

Thus, also when considering Yoshida et al., "A reference may be said to teach away when a person of ordinary skill, upon reading the reference, would be discouraged from following the path set out in the reference, or would be led in a direction divergent from the path that was taken by the applicant." *In re Gurley*, 27 F.3d 551, 553 (Fed. Cir. 1994). Again, in the instant case, the whole of the teachings of Yoshida et al. show that PML/RAR $\alpha$  is degraded via the proteasome pathway, i.e. a non-lysosomal pathway. Thus, Yoshida et al. unquestionably teach away from doing what Applicants have done; i.e., determining whether an agent destabilizes lysosomes and increases lysosomal-dependent PML/RAR $\alpha$  protein degradation. Accordingly, there would be no motivation to combine this reference with the

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teachings of Bard *et al.* for determining whether an agent destabilizes lysosomes, in particular considering the teachings of Bard *et al.* with regard to toxicity associated with lysosomal degradation and the property making an agent not useful clinically.

Based on the deficiencies in teaching of the combined references concerning the use of doses that are clinically useful, and the fact that the references each teach away from the instant method, Applicants respectfully assert that the basis of this rejection is not supported by the whole of the teachings of the cited references. Thus, the combined teachings of Bard *et al.* and Yoshida *et al.* cannot be held to make the present invention of amended claim 8 obvious under 35 U.S.C. 103(a). It is therefore respectfully requested that this rejection be reconsidered and withdrawn.

## II. Conclusion

Applicants believe that the foregoing comprises a full and complete response to the Office Action of record. Accordingly, favorable reconsideration and subsequent allowance of the pending claims is earnestly solicited.

Respectfully submitted,



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